Two new genera and species of sponges (Porifera, Demospongiae) without skeleton from a Mediterranean cave

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ABSTRACT
Two new genera and species of Demospongiae are described from a northwestern Mediterranean littoral cave characterized by cold homothermy, which shelters deep-sea invertebrates. The two new sponges have neither mineral nor fibrous skeleton. Their cytology is described using transmission electron microscopy. *Thymosiopsis cuticulatus* n.g. n.sp. (Chondrillidae) shares some characters with *Thymosia* Topsent, but lacks the diagnostic spongin fibres. *Myceliospongia araneosa* n.g. n.sp. has unusual anatomy, cytology, and mode of growth. No clear relationship with any order of the Demospongiae is indicated and the sponge is classified as *incertae sedis* within the Demospongiae.

KEY WORDS
Porifera,
taxonomy,
Mediterranean,
new genera and species,
cave.

RÉSUMÉ
Deux nouveaux genres et espèces d'éponges (Porifera, Demospongiae) sans squelette d'une grotte méditerranéenne. Deux nouveaux genres et espèces de Demospongiae sont décrits d'une grotte littorale de la Méditerranée nord-occidentale, qui est caractérisée par une homothermie froide et abrite des invertébrés de mers profondes. Les deux nouvelles éponges sont dépourvues de squelette minéral ou fibreux. Leur cytologie est décrite en microscopie électronique. *Thymosiopsis cuticulatus* n.g. n.sp. (Chondrillidae) a des affinités avec *Thymosia* Topsent, mais ne possède pas les fibres de spongine distinctives. *Myceliospongia araneosa* n.g. n.sp. est très inhabituelle par son anatomie, sa cytologie et son mode de croissance. Aucune relation ne peut être établie avec un ordre de Demospongiae et l'éponge est classée comme *incertae sedis* dans les Demospongiae.

MOTS CLÉS
Porifera,
taxonomie,
Méditerranée,
nouveaux genres,
nouvelles espèces,
grotte.

INTRODUCTION

Sponges without skeleton, whose taxonomy is especially difficult in the absence of the conventional diagnostic characters, display a remarkable abundance and variety in Mediterranean submarine caves. Representatives of all the described genera: *Oscarella* Vosmaer, 1884 and *Pseudocorticium* Boury-Esnault *et al.*, 1995 (Homoscleromorpha, Plakinidae), *Chondrosia* Nardo, 1847 (Tetractinomorpha, Chondrillidae), *Hexadella* Topsent, 1896 (Ceractinomorpha, Darwinellidae), and *Halisarca* Dujardin, 1838 (Ceractinomorpha, Halisarcidae) are present in Mediterranean caves, either in semi-obscure zones near the cave entrance or in darkest recesses. Recent studies have shown that at least four different species of *Oscarella* could be present in a single cave (Muricy *et al.* 1996), two of them known exclusively from dark caves. The recently described genus *Pseudocorticium* is as yet known only from caves (Boury-Esnault *et al.* 1995). This abundance of sponges without skeleton in caves probably reflects the situation on vertical and overhanging surfaces of steep cliffs in bathyal environments, with which caves have faunistic similarities (Harmelin *et al.* 1985; Vacelet *et al.* 1994). The species which are known only from caves most likely also live on the continental slope, especially in steep canyons where they are as yet unrecorded due to the obvious difficulties in observation and sampling.
These sponges usually have close relatives which have a skeleton, so their taxonomic position is seldom disputed. An exception, however, is the genus *Halisarca*, with no known skeletonized relatives and an unusual anatomy. After having been classified for a long time either as *incertae sedis* or in the order Dendroceratida, the genus has finally been isolated in the new order Halisarcida (Bergquist 1996).
We describe here, two new sponges without skeleton from the dark zones of a cave which shelters an unusually high number of deep-sea invertebrates, due to a stable homothermic regime around 13 to 14.5 °C, similar to that of the deep Mediterranean (Vacelet *et al.* 1994). The new sponges, which are unrecorded in caves with temperature variations similar to those of the littotal zone, are probably representatives of the undescribed sessile fauna of the deep-Mediterranean canyons. They belong to two new genera, one of which has uncertain affinities. Their description includes data on ultrastructural cytology, which are particularly important in the absence of the conventional taxonomic characters of the skeleton.

MATERIALS AND METHODS

Field observations and sampling

The specimens were observed *in situ* in the "3PP" cave near La Ciotat (43°09.47'N - 05°36.01'E) with an underwater magnifying lens (Mladenov & Powell 1986) and photographed with a close-up lens. Pieces of specimens were collected, either by scraping sponges from the substratum or by detaching fragments of the cave walls. Specimens, except those used for detailed microscopy (see below) were fixed in formalin and stored in alcohol.
A general description of the cave is given in Vacelet *et al.* (1994) and Vacelet (1996). Temperature recordings were made over two years using Deep-Sea Sealoggers thermographs (Vacelet 1996; Harmelin 1997).

Cytology

For light and transmission electron microscopy (TEM), the specimens were fixed *in situ* in glutaraldehyde 2.5% in a mixture of 0.4 M cacodylate buffer and sea water (4 vol.: 5 vol.). They were maintained in the fixative for 24 hours and post-fixed 2 hours in 2% osmium tetroxide in sea water. Specimens were decalcified in 10% RDO (Du Page Kinetic Lab) in sea water in order to remove the underlying substratum, dehydrated through an alcohol series and embedded in Araldite. Semi-thin sections were stained with toluidine blue. Thin sections, contrasted with uranyl acetate and lead citrate, were observed under a Zeiss EM 912 transmission electron microscope.

SYSTEMATICS

Order CHONDROSIDA
Boury-Esnault *et* Lopes, 1985
Family CHONDRILLIDAE Gray, 1872

Thymosiopsis n.g.

TYPE SPECIES. — *Thymosiopsis cuticulatus* n.sp.

ETYMOLOGY. — The genetic name is derived from *Thymosia*, a genus of the same family and the suffix *-ops* (in Greek: with the aspect of).

DIAGNOSIS
Encrusting Chondrillidae. General organization similar to that of the genus *Thymosia*, having a smooth surface, a superficial cuticle and pore-sieves, a marked cortex enriched with fibrillar collagen, but lacking spongin fibres.

Thymosiopsis cuticulatus n.sp.
(Figs 1-3)

TYPE MATERIAL. — **North-Western Mediterranean.** La Ciotat, 3PP cave. — 50 m from cave opening, 16 m in depth, 7.VII.1996: holotype, 1 fragment (MNHN D JV 59). — 30 m from cave opening, 20 m in depth, 3.III.1997: paratype, 2 fragments (MNHN D JV 60).

ETYMOLOGY. — The species name refers to the presence of a cuticle (from *cuticula*, Latin, thin skin).

LOCALITY AND HABITAT. — Known only from 3PP cave, 1.2 km south-west of La Ciotat on the French Mediterranean coast (43°09.47'N - 05°36.01'E). The sponge has been found on vertical or overhanging walls, 16 to 22 m deep, 30 to 80 m from the cave opening, in a trapped body of water whose temperature varies from 13 to 14.5 °C year round (Vacelet *et al.* 1994). The sponge is not very common in the cave and only a few large specimens have been observed.

DESCRIPTION
Shape and size
The sponge is encrusting, up to 15/20 cm, and 3 to 5 mm thick in the centre, thinner on the edges which are irregular (Fig. 1A). The sponge is firmly attached to the substratum by its whole undersurface, and insinuates into small cavities such as empty serpulid tubes.

Colour
The *in situ* colour is white or yellowish white. A faint brown tinge due to a thin, inconstant deposit of iron and manganese oxides on the cuticle, is visible in places, especially on ridges corresponding to the irregularities of the substratum. Thinner parts of the sponge, mostly on the edges, are greyish due to the black underlying rock seen through the transparent tissue. After fixation in formalin, the alcohol-preserved specimens are whitish.

Surface
The surface is smooth, but irregular as the sponge closely follows the irregularities of the substratum. Small apertures, approximately 0.6 mm in diameter (measurements from underwater close-up photographs of non-contracted specimens) are gathered in oval or circular depressions with an elevated outline, 5 to 15 mm in diameter (Fig. 1A). Most of the depressions are probably inhalant pore-sieves. Superficial canals, visible below the ectosome especially on the thin zones at the periphery of the sponge, converge towards the larger of these depressions, which may be composite oscules. Single oscules have not been observed, neither *in situ* nor on collected specimens. The holotype, which was living relatively near the cave entrance (30 m), was covered by various encrusting sponges, bryozoans and didemnids, which were loosely attached on the surface between the pore-sieves. Most of the specimens, however, were free of macroepibionts.

Texture
The consistency is quite cartilaginous, although easy to tear.

Skeleton
There is neither spicule nor spongin fibre skeleton. A small amount of foreign material is frequently included in the choanosome.

Anatomy and cytology
Ectosome and choanosome are clearly distinct (Fig. 1B), although the ectosome is not detachable. The ectosome is 40-50 µm deep and is lined on the outer surface by a thin, non-cellular cuticle, which appears, in sections observed with

the light microscope, as a wrinkled layer, 2 µm in thickness. This cuticle is covered in most places by a mucous layer metachromatically stained by toluidine blue. In TEM (Fig. 2A), the surface appears to be covered by a wavy dense layer, 0.15 µm in thickness, in direct contact with the collagen fibrils of the underlying tissue, without any pinacoderm. This thin layer is covered by a zone of very dark, irregular granules, which are probably deposits of iron and manganese oxides, frequent in caves (Harmelin *et al.* 1985; Bianchi *et al.* 1986). The oxide deposit, visible as a brownish coloration in some areas of the sponge surface, and the presence of epibionts on some specimens

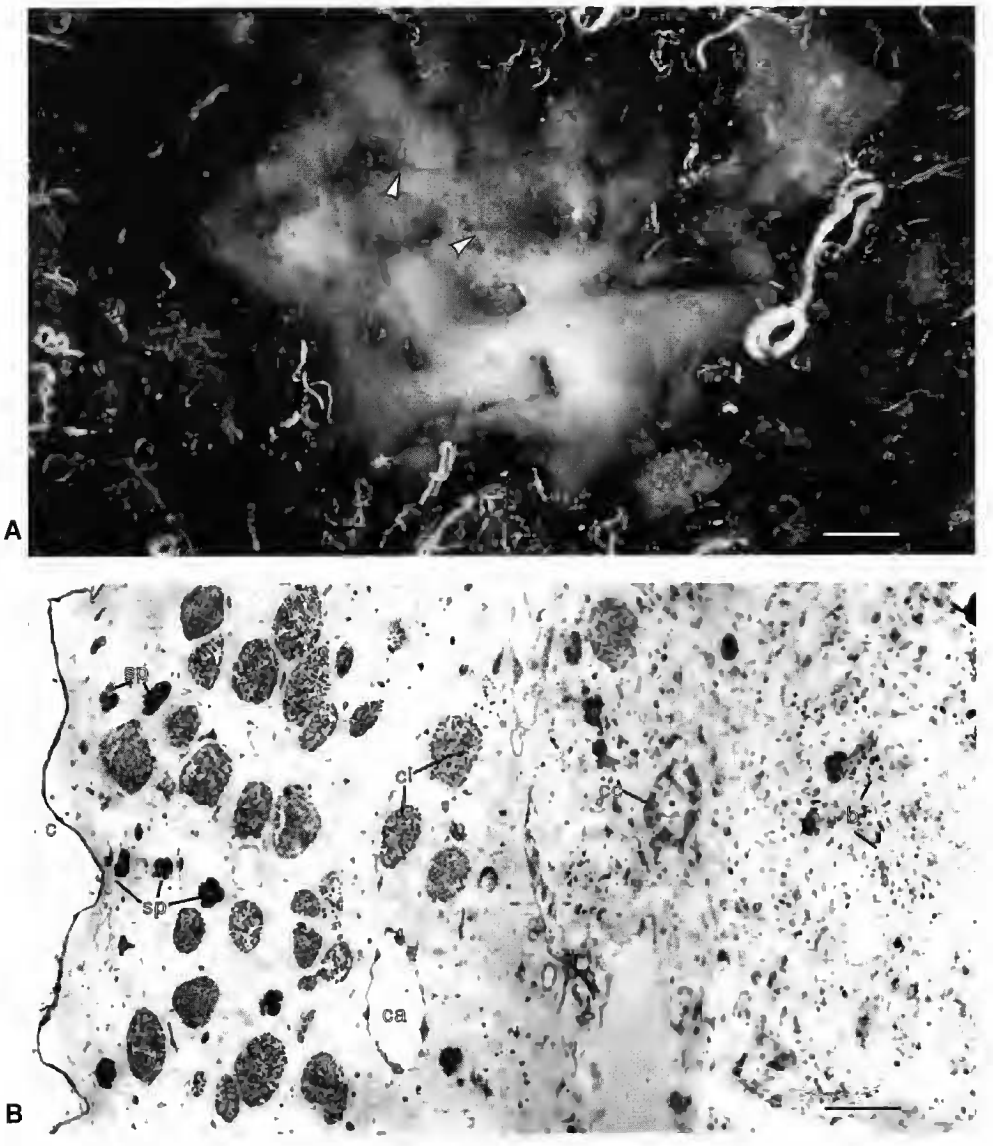


FIG. 1. — *Thymosiopsis cuticulatus* n.g. n.sp., **A**, specimen *in situ*, arrow head: pore-sieves; top right, the sponge with superficial canals is *Diplastrella bistellata*; **B**, semi-thin section showing the tissue organization. **b**, extracellular bacteria; **c**, cuticle; **ca**, canal; **cc**, choanocyte chamber; **ci**, cells with inclusion Types 1 and 2; **sp**, spherulous cells Type 3. Scale bars: A, approximately 13 mm; B, 16 µm.

both indicate that the cuticle is not transient, but remains stable for at least several months. This layer is covered by a fibrillar area, 1.5 to 2 µm in thickness, having the appearance of a mucous sheath. An empty space is often present between the wavy dark layer and the other layers.

The ectosome contains bundles of collagen fibrils, most of which are parallel to the surface. The fibrillar bundles are thin (1-3 µm), not very densely packed, and the ectosome is considerably thinner and less specialized than in sponges with a true cortex, such as *Chondrosia* or *Tethya*. The ectosome also contains spherulous cells (Type 3, described below), large cells with irregular, small granules (Types 1 and 2), and extracellular bacteria.

In the choanosome, collagen fascicles are less dense. Choanocyte chambers occur in relatively low density. Most of the choanosome volume is occupied by Types 1 and 2 cells, closely pressed together in places, and by a high number of extracellular symbiotic bacteria.

Exopinacocytes were not observed. They are absent in the areas lined by the cuticle, and are probably found only in the pore-sieves, for which we have no good sections. Endopinacocytes are not flagellated (Fig. 2B).

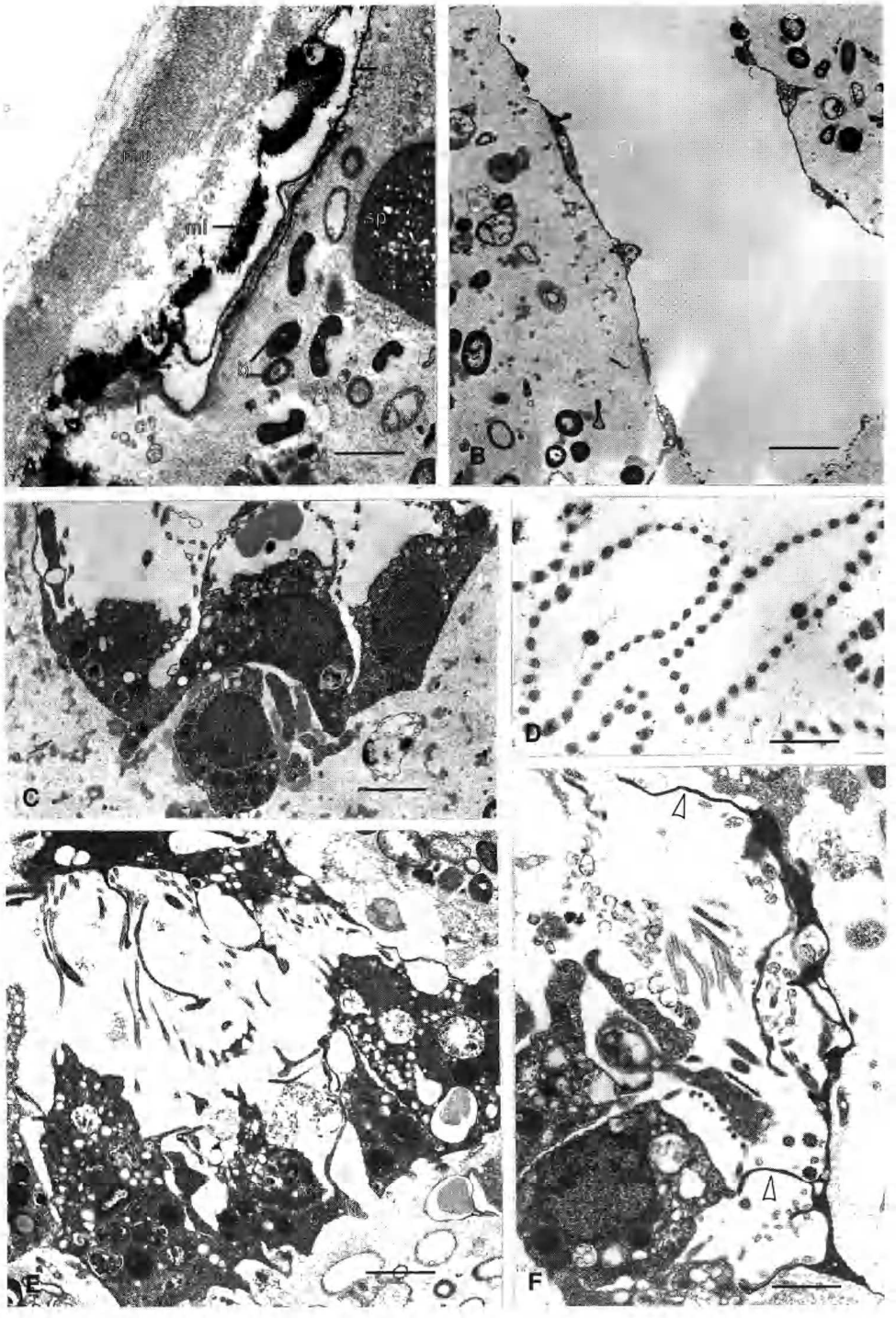
Choanocyte chambers (Figs 1B, 2C-F) are eurypylous and spherical, 15 to 30 µm diameter. Two aspects have been observed. In some chambers (Fig. 2C), choanocytes are cylindrical or pyramidal, 3 to 4 µm in size with a nucleus approximately 2 µm in diameter, spherical or pyriform, rarely nucleolated, and a cytoplasm containing few phagosomes. In other chambers (Fig. 2E, F), choanocytes are very irregular, and their cytoplasm contains a larger number of phagosomes. In both cases, the collar is 3.6 µm in diameter and made up of thirty-two to thirty-four microvilli (Fig. 2D). There is no periflagellar sleeve. The flagellum possesses two thin lateral extensions, which are faintly visible on some transverse sections and which may be interpreted as poorly preserved flagellar vanes (Afzelius 1961; Mehl & Reiswig 1991). Conventional apopylar (or cone) cells (Boury-Esnault *et al.* 1984; Langenbruch *et al.* 1985; De Vos *et al.* 1990) and central cells (Reiswig & Brown 1977) were not observed. The choanocyte base is flat or displays a few short pseudopodia anchoring the cell in the underlying mesohyl, usually without a pinacocyte lining. However, in certain chambers with irregularly-shaped choanocytes, very dense fusiform cells line the choanocyte base and extend long, thin lamellipodia which insinuate between the choanocyte bodies and ramify into the chamber cavity. Lamellipodia are in close contact with the choanocyte collar or flagellum (Fig. 2F). These cells, which are remarkable in the density of their cytoplasm, seem to be pinacocytes lining the choanocyte base, as observed in some demosponges, but in this case possibly having a role in the regulation of the water flow by extending pseudopodia into the chamber lumen.

Four distinct types of cells with inclusions are present:

Type 1. (Fig. 3A) Large cells, 15 to 18 µm in diameter, containing numerous granules enclosed in clear vesicles delineated by a thin sheet of cytoplasm. The cells have a degenerating aspect, with nucleus and organelles rarely observed. The granules, 0.5 to 1.1 µm in diameter, have a finely granular content and an irregular outline, with a cloudy aspect. They appear moderately dense to the electrons in TEM and are stained by toluidine blue in semi-thin sections. Type 1 cells are abundant by places in the choanosome and occur as dense clusters in the ectosome.

Type 2. (Fig. 3B) Large cells, approximately the same size as Type 1, with similar clear vesicles containing a dispersed fibrillar material and small rod-like inclusions, 0.1-0.2/0.45-1.1 µm, with a clear central area surrounded by an irregular dense zone. These rod-like inclusions are probably bacteria, morphologically different from the extracellular ones described below. These cells, whose cytoplasmic outline is rarely observed, appear to be advanced stages in degeneration of Type 1 cells.

Type 3. (Fig. 3C) Spherulous cells, approximately 10 µm in diameter, with two to ten large, homogeneous spherules, 1 to 4.5 µm in diameter, which occupy most of the cell volume. Spherules were occasionally observed free in the mesohyl, especially in the ectosome, after degeneration of the cell. However, these cells are not clearly secreting the intercellular matrix, as they do in some *Halisarca* or in *Chondrosia* (Vacelet & Donadey 1987).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Type 4. (Fig. 3D) Microgranular cells, most often elongated (2.5-3 µm/4.5-8 µm) with dense, ovoid inclusions, 0.3/0.9 µm in the cytoplasm. These cells are not vety abundant.

The collagen fibrils are thin, approximately 15 nm in diameter (Fig. 3B). Their periodicity is not apparent. They seem to belong to the smooth type of collagen fibrils (Garrone 1978), although this character is not well defined here. They are organized in bundles, 1 to 3 µm in diameter, especially in the ectosome. There is no disjunction between the collagen bundles and a granulo-fibrillar matrix such as is observed in some species of *Halisarca* or of Chondrillidae (Vacelet & Donadey 1987).

Symbiotic extracellular bacteria (Figs 2A, B, 3D) found in the mesohyl belong to several different morphological types, varying in size from 0.2-0.4 µm to 0.8-2.5 µm. They display the various morphologies found in demosponges having a high density mesohyl (Vacelet 1975; Vacelet & Donadey 1977; Boury-Esnault *et al.* 1995), in which the cell walls are complex and frequently display a remarkable enlargement of the periplasm. An unusual type (Fig. 2A) is a rod-like cell, 0.4 µm in diameter and at least 1.5 µm in length, with a contorted shape, dense cytoplasm and reduced nuclear area, which is rarely observed in other demosponges, although it morphologically resembles the intra- and intercellular bacteria described in the parenchymella of *Haliclona tubifera* (Woollacott 1993).

Reproduction

Not observed.

REMARKS

This sponge appears to have affinities with *Thymosia* Topsent, 1895, a monospecific genus of "keratose" sponge from the North-East Atlantic. After various allocations, the genus is presently classified in family Chondrillidae (= Chondrosiidae), as originally proposed by Topsent. The main differences between *Thymosia guernei* Topsent, 1895 as recently redescribed (Boury-Esnault & Lopès 1985; Rosell 1988; Carballo 1994), and *Thymosiopsis cuticulatus* n.sp. are the absence of the verrucose horny fibres which are highly diagnostic of *Thymosia*, and the composite nature of the oscules. The two sponges share the presence of a cuticle on most of the surface, of pore-sieves and of a specialized ectosome constituting a weakly developed cortex. The characters of the choanocyte chambers, aquiferous system and Type 3 cells with inclusions (spherulous cells) cannot provide a diagnosis, but do not contradict the supposed affinity between the two genera.

These similarities could be differently interpreted. The unusual fibres of *Thymosia guernei* have been interpreted as a hydroid skeleton (Betgquist 1980). The new sponge could be considered either as specimen of *Thymosia guernei* without the hydroid associate, or as a Mediterranean *Thymosia* – a genus never recorded from this sea – which would have lost the spongin fibres in low-energy environments such as the deep sea or a cave. Both hypotheses appear unlikely. The hydroid nature of the fibres, already ruled out by Topsent (1895) in the original description, has been contradicted by all recent records of the sponge. We have checked their genuine natute on new specimens of *Thymosia guernei* from Portugal (unpublished observations). The ultrastructure of these specimens shows that, although the general organization is rather similar, the two sponges differ clearly by the structure of cells with inclusions. Most significantly, cells with inclusions Type 1 and Type 2, which are very abundant and remarkable in *Thymosiopsis cuticulatus*, are absent in *Thymosia guernei*. These differences indicate that the two sponges belong to different species. The absence of the characteristic fibres, combined with these differences in cytology, justifies the creation of a new genus, parallel to the present classification in the Darwinellidae where the genus *Hexadella*, without skeleton, is considered distinct from *Aplysilla* and *Darwinella*.

FIG. 2. — *T. cuticulatus* n.sp., **A**, transmission electron micrograph (TEM) of the sponge surface; **b**, bacteria; **c**, cuticle; **mi**, mineral deposit; **mu**, mucous deposit; **sp**, isolated spherule of a spherulous cell. **B**, TEM of the choanosome showing a small canal; **C**, TEM of a choanocyte chamber. **D**, TEM of a choanocyte collar, note the flagellar vanes. **E**, TEM of a choanocyte chamber with irregularly-shaped choanocytes. **F**, TEM of a choanocyte chamber showing an elongate cell with dense cytoplasm extending lamellipodia in the chamber lumen (arrows). Scale bars: A, 1.6 µm; B, 2.5 µm; C, 2.0 µm; D, 1.0 µm; E, 2.0 µm; F, 1.3 µm.

The obvious differences in the development of cortex and of collagen bundles between the new sponge and the genus *Chondrosia* (Garrone *et al.* 1975) precludes its allocation to that genus. The distinction of *Thymosiopsis* from *Chondrosia* is also justified by differences in the canal system organization (Schulze 1877; Bavestrello *et al.* 1988), and by the presence of a constant cuticle and well organized pore-sieves in *Thymosiopsis*. The anatomy and cytology of the new sponge are certainly more similar to those of *Thymosia* than of *Chondrosia*.

The genus *Thymosia* is rather puzzling in the Demospongiae, where it has been diversely allo-

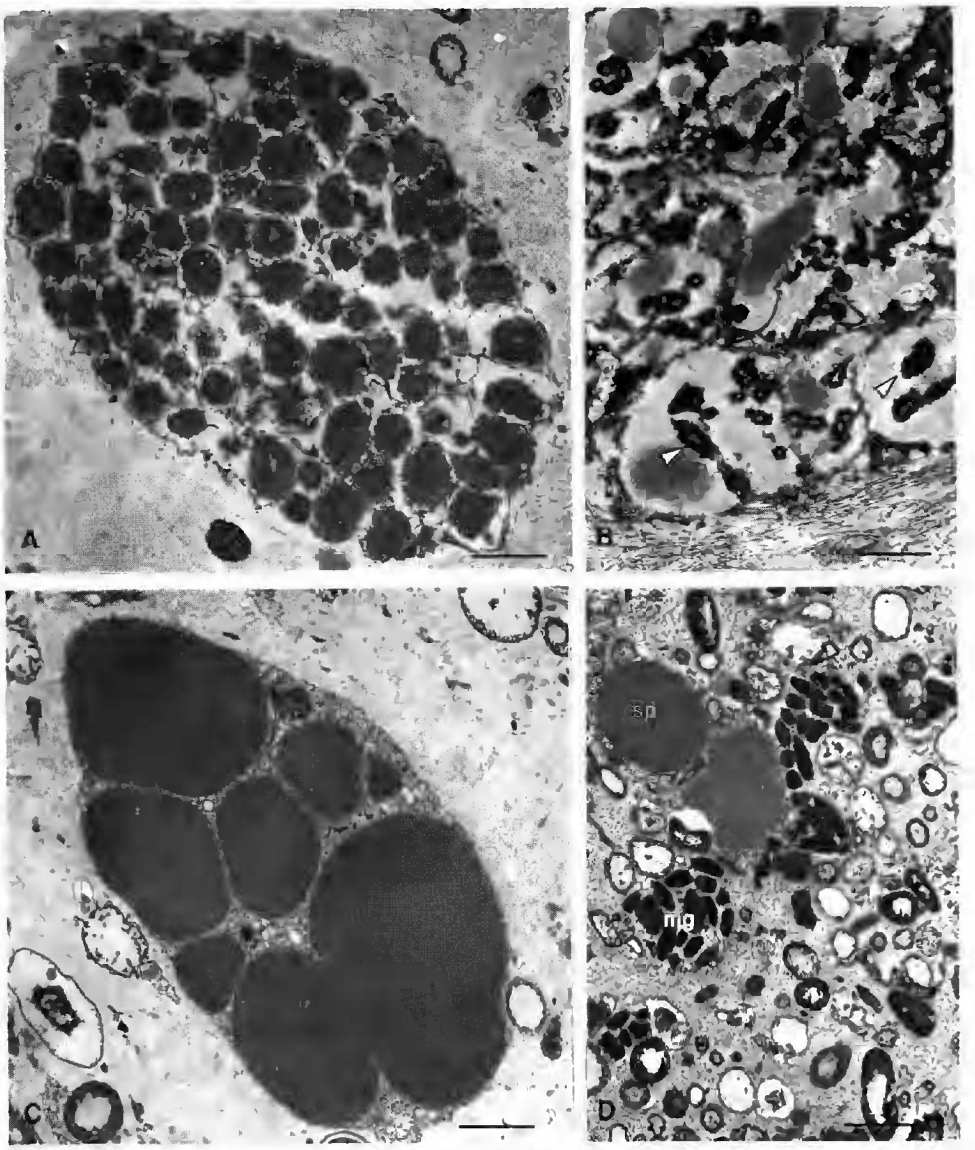


FIG. 3. — *T. cuticulatus* n.sp., **A**, TEM of a granular cell (Type 1 cell); **B**, TEM of a granular cell (Type 2 cell) with intracellular bacteria (arrows). **C**, TEM of a spherulous cell (Type 3 cell). **D**, TEM of the mesohyl showing various symbiotic bacteria, a degenerating spherulous cell (Type 3 cell, **sp**) and degenerating microgranular cells (Type 4 cell, **mg**). Scale bars: A, 1.3 µm; B, 0.8 µm; C, 1.3 µm; D, 1.6 µm.

cated (Rosell 1988). It bears unusual, nodulose spongin fibres and has cytological characters different from those of "keratose" sponges. Most authors presently classify the genus in family Chondrillidae (= Chondrosiidae), because of anatomical resemblances with the genus *Chondrosia*. These resemblances are not fully convincing, especially as the ectosome is less specialized than in *Chondrosia* or in *Chondrilla*, and does not constitute a thick cortex with dense fascicles of collagen. Furthermore, *Thymosia guernei*, which possesses pore-sieves which are considerably more organized than the cribriporal chones of the Chondrillidae (Schulze 1877; Bavestrello *et al.* 1988), does not display the disjunction between both cellular and collagen elements and a granulo-fibrillar matrix as described in Chondrillidae (Vacelet & Donadey 1987). In our opinion, the relationships of *Thymosia* and *Thymosiopsis* with the Chondrillidae remain to be confirmed; until this can be done, the genera should remain in that family.

DEMOSPONGIAE, Order *incertae sedis* Family *incertae sedis*

Genus *Myceliospongia* n.g.

TYPE SPECIES. — *Myceliospongia araneosa* n.sp.

ETYMOLOGY. — The generic name derives from *mukés* (in Greek: fungus) and refers to the shape of the type species.

DIAGNOSIS

Demospongiae *incertae sedis* without skeleton and without cortex. Body encrusting, from which arises a reticulation of thin filaments covering the substratum. The sponge has a reduced canal system and a low number of choanocyte chambers. Exopinacocytes non flagellated, covered by a mucous sheet. Most of the cells contain granular inclusions and symbiotic bacteria.

Myceliospongia araneosa n.sp. (Figs 4-6)

TYPE MATERIAL. — **North-Western Mediterranean.** La Ciotat, 3PP cave 60 m from cave opening, 18 m in depth. — 7.VII.1996: holotype (MNHN D JV 61). — 17.XII.1996 : paratype (MNHN D JV 62).
Holotype and paratype are fragmentary specimens.

ETYMOLOGY. — The species name derives from *araneosus* (in Latin: similar to a spider web), and refers to the shape of the margin of the sponge.

LOCALITY AND HABITAT. — Known only from 3PP cave, 1.2 km south-west of La Ciotat on the French Mediterranean coast (43°09.47'N - 05°36.01'E). The sponge lives on vertical or overhanging walls, 18 to 21 m deep, 50 to 80 m from the cave opening, in a trapped body of water whose temperature varies from 13 to 14.5 °C year round. It seems to be absent farther in the cave, which extends up to 120 m from opening. The sponge is not very common in the cave and only a few large specimens have been observed. However, specimens may easily be overlooked as they are occasionally covered by other sponges.

DESCRIPTION

Shape and size

Sponge encrusting, approximately 1 mm in maximum thickness, composed of a "body" covering most of the surface of the substratum, although with irregular lacunae, and of filaments which extend a long distance from the body and are closely applied to the substratum (Fig. 4A). The maximum size observed is 25 cm in diameter for the body, with the filaments visible *in situ* extending at least 12 cm from the body, the total diameter of the surface colonized by the sponge thus being approximately 50 cm. The filaments decrease in diameter with the distance from the body. They can be extremely thin at their extremities: filaments 5 µm in diameter extending to 20 µm in length have been observed. They divide dichotomously or anastomose, to form an irregular reticulation. The body of some specimens is entirely covered by other sponges, predominantly *Pachastrissa pathologica* (Schmidt, 1868) or *Rhaphisia laxa* Topsent, 1892 in which case only the filaments are visible. The filaments most often cover the rocky surfaces, following the irregularities of the substratum or insinuating into small cavities such as empty serpulid worm tubes. They may also run on the surface of other sponges. They differ from the stretched filaments described in some Chondrillidae or Homoscleromorpha living under sledges, which

are elongated areas of sponge tissue stretched by the weight of a detached piece of substratum (Gaino & Pronzato 1983). The sponge is not firmly attached and can be easily removed from the substratum.

This aspect has been observed year round. Visual observations over two years and the comparison of serial underwater photographs of the same individual during a six months period from July to December (Fig. 4B, C) indicate a rather stable situation over time, with a slight increase of the surface covered by the sponge. The filaments and body maintained the same general shape over six months. Some of the filaments increased in diameter and coalesced, and a small zone which was colonized by a loose reticulation in July, was wholly covered in December. Conversely, a few filaments regressed, but this was less common. The spider-web like reticulation on the rock around the sponge consequently does not correspond to a rapid regression or a fragmentation process, but rather to a slow growth process of a sponge maintaining this special shape over long periods.

Colour

Colour is white in life. Preserved specimens are cream coloured. Some specimens, especially the holotype, have turned clear pink in alcohol after fixation in formalin.

Surface

The surface is smooth, appearing sometimes irregular because the sponge closely follows the irregularities of the substratum. There is no detachable ectosome. The oscules, 0.5 to 1.2 mm in diameter (measurements from underwater close-up photographs), are rare on the body and absent from the filaments. There is no pore-sieve, and the ostia are not visible. Canals, approximately 1 mm in maximum diameter, are visible under the ectosome in the body and in the largest filaments, with only one canal in each filament.

Texture

Texture is fleshy, rather soft and fragile.

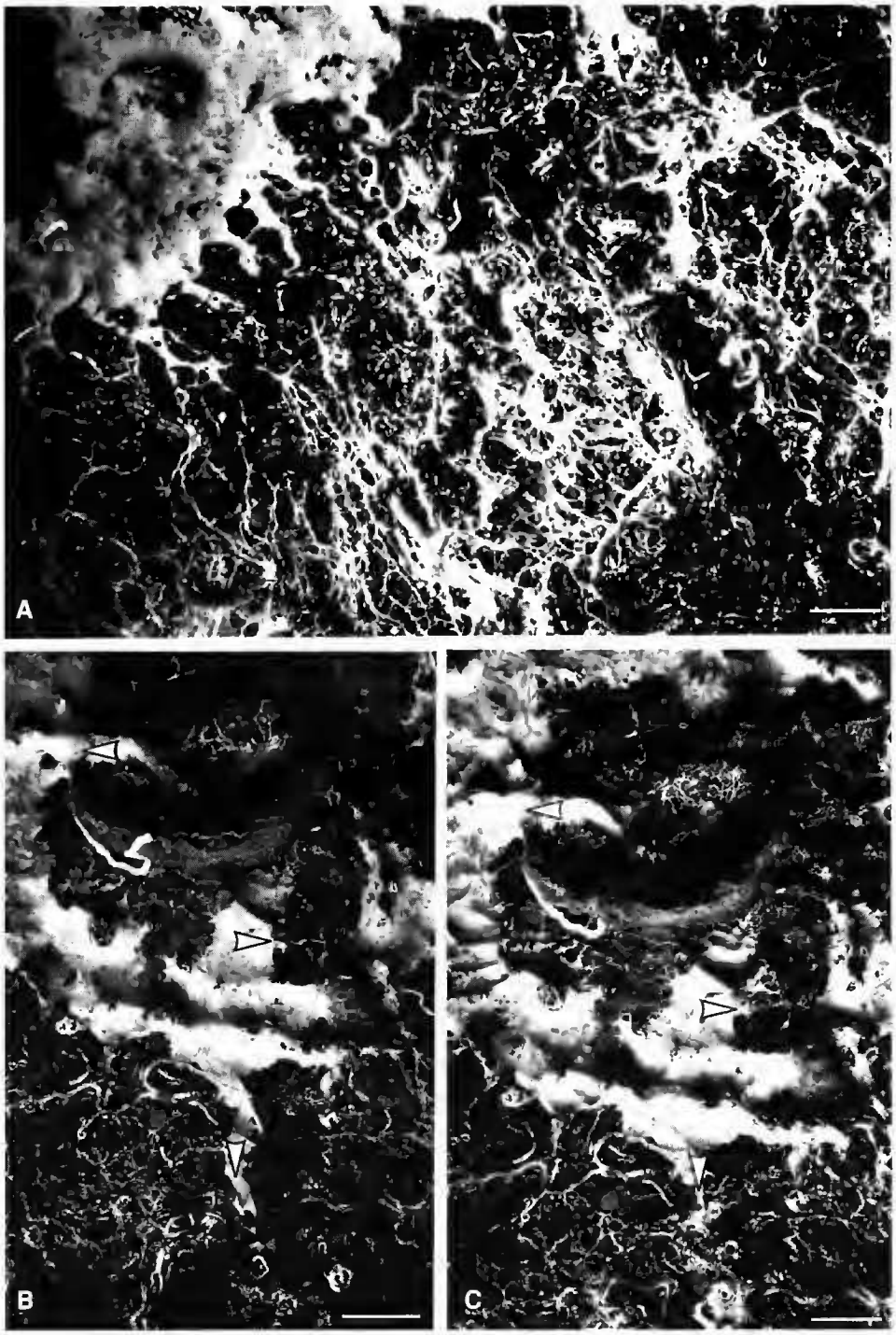
Skeleton

Spicule, spongin fibre, bundle or thick condensation of collagen fibrils are absent.

Anatomy and cytology

The dermal structure (Fig. 5A, B) consists of a thin ectosome, with a layer of T-shaped exopinacocytes and a thin cuticle, without any underlying special ectosome differentiation. Exopinacocytes have no flagellum. Their cytoplasm contains dense granules similar to those of the granular cells of the mesohyl, although smaller (0.4 to 1.1 µm) and less numerous. Vacuoles with symbiotic bacteria are absent. The lateral expansions of the exopinacocytes are frequently superposed for a few micrometres, and are linked without specialized cell junctions. The thickness of the pinacocyte expansions is variable, from very thin sheets without any inclusions to swellings, 3 to 4 µm thick, containing dense granules. The outer surface of the exopinacocytes is covered by an extremely thin cuticle, covered by a well organized fibrillar material, which is quite similar to the layer termed "glycocalyx" by Willenz (1981, 1982) in *Hemimycale columella* Burton, 1934. This outer cover of the pinacoderm, 0.12 to 0.25 µm in total thickness, is made up of a clear zone, containing fibrils mostly perpendicular to the outer cell membrane of the pinacocyte, followed by a dense zone which is covered by thin erect fibrils, which are denser at their extremities. Contrary to *Hemimycale columella* there are no bacteria fixed on this external "glycocalyx", although bacteria are occasionally engulfed. This structure is found on the outer surface of both body and filaments of the sponge. In places, the exopinacoderm displays invaginations, approximately 2 to 4 µm in diameter, leading to small canals (Fig. 5A). The canals are lined by pinacocytes which have a similar "glycocalyx", but which are more ovoid in shape and with a nucleus 2.8 to 3 µm in diameter. These

FIG. 4. — *Myceliospongia araneosa* n.g. n.sp., **A**, specimen *in situ*; **B**, *in situ* view, 11.VII.1996; **C**, same as figure B, 3.XII.1996; although the surface covered by the sponge slightly increased (horizontal arrows), most filaments remained stable, rearrangement occurred in a few places (vertical arrows). Scale bars: A, approximately 13 mm; B, approximately 12 mm; C, 12 mm.


A
B
C

openings are probably ostia, although of an unusually small size.

The undersurface in contact with the substratum is made up of ovoid basopinacocytes which are lined by a cuticle similar to thar of the exopinacoderm, although it appears devoid of a "glycocalyx" layer.

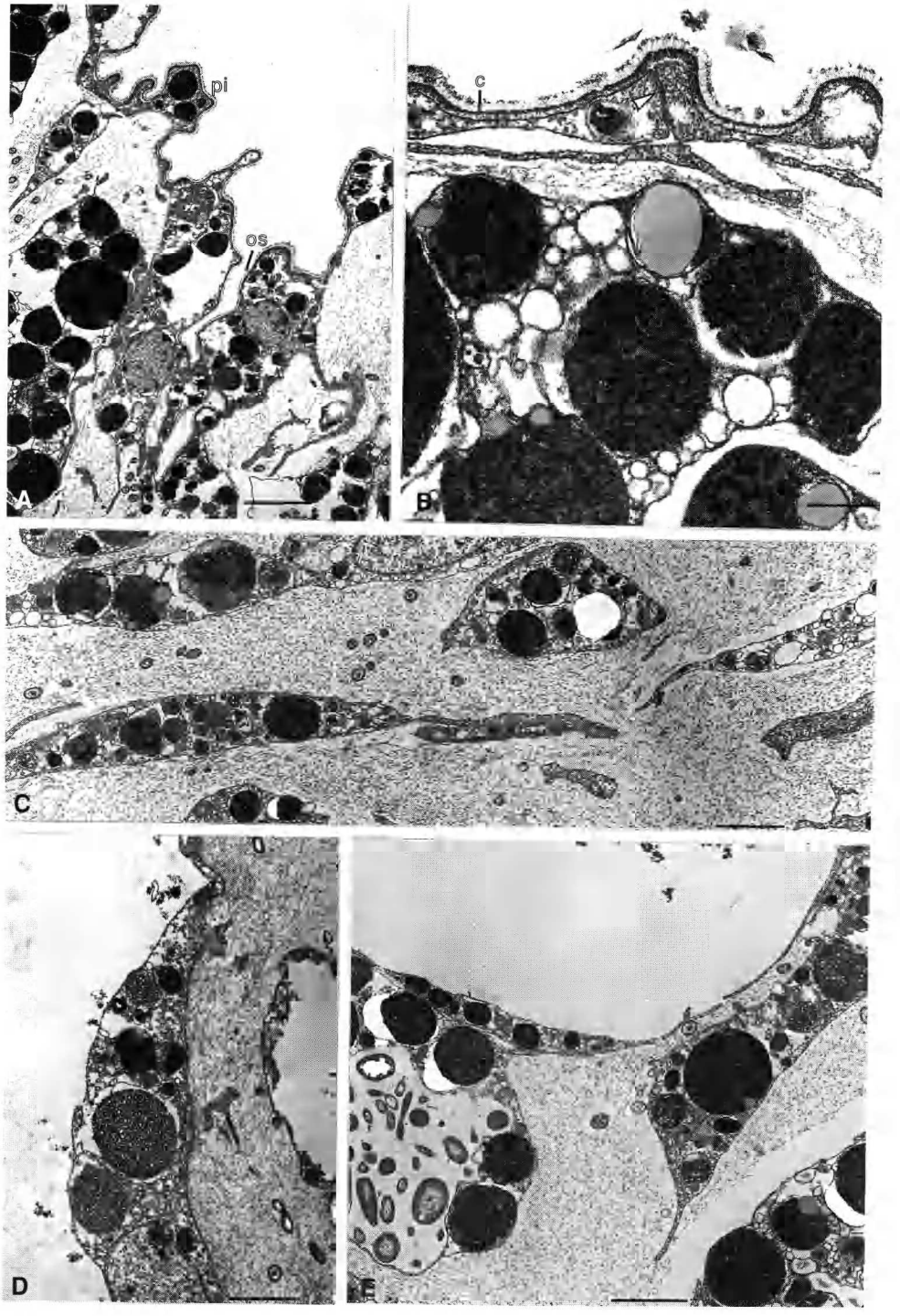
The choanosome also shows a very unusual structure. Canals and choanocyte chambers (Fig. 6A) are rare in sections. The canals visible in the *in situ* specimens are small and difficult to observe in preserved specimens, where they are probably contracted. They are lined by ovoid endopinacocytes, with a nucleus of 1.9 to 2.5 µm in diameter, without flagellum and containing dense granules similar to those of the exopinacocytes (Fig. 5D, E). The endopinacocytes are devoid of the granulo-fibrillar layer ("glycocalyx") present on the exopinacocytes. Most of the choanosomal tissue is made up of a single cell category, *i.e.* granular cells of highly diverse size and shape, containing a variable amount of dense inclusions and intracellular symbiotic bacteria. Spherulous cells are absent.

Granular cells (Fig. 5A-C, E), with a frequently nucleolated nucleus 2.4-4 µm in diameter, may be up to 40 µm in maximum diameter when ovoid or spherical. They contain a variable amount of spherical inclusions which are highly variable in size from 0.2 to 12 µm in diameter. Inclusions, which are surrounded by a membrane, contain a large mass made up of dense ovoid granules, 0.1 µm in maximum size, included in a dense matrix. This mass, usually spherical, is often deformed by a few lipid globules or irregular, myelinic-like granules. These inclusions are intensely metachromatically stained purple by roluidine blue in semi-thin sections. The large granular cells also contain symbiotic bacteria included in vacuoles of variable size, which often occupy most of the cell volume, and stain clear blue with toluidine blue (Figs 5E, 6C, D). The bacreria belong to several different morphological types, from rod-like bacteria (0.15/1.7 µm) to ovoid cells (0.8/1.7 µm, rarely up to 1/3.7 µm). They display the various morphologies found in demosponges having a high density mesohyl (Vacelet 1975; Vacelet & Donadey 1977; Boury-Esnault *et al.* 1995). Most of them have a complex cell wall. Cells of Type E (Vacelet 1975), with an enlarged periplasm bearing an indentation, are frequent.

The shape of the granular cells varies importantly according to the zone of the mesohyl, from spherical to very elongated cells. The elongated cells, which have smaller and clearer granules, no bacteria, and long pseudopodia, are usually in parallel arrangement in tracts, especially in the filaments, suggesting intense, orientated cell migrations along trails with denser and polarized collagen fibrils (Figs 5C, 6B). Intermediate stages between elongated cells and spherical cells are numerous.

Choanocyte chambers (Fig. 6A) are present in low density, and only a few of them have been observed. They are ovoid, approximately 7.5 to 15/20 to 24 µm. The choanocytes have a cylindrical (2.5 µm in maximum height) or flattened body, with a spherical, anucleolate nucleus 1.6 to 1.8 µm in diameter. Their base displays lateral lamellipodia, up ro 5.6 µm long, which attach ro or cover the appendages of the other choanocytes. The choanocyte chamber is thus surrounded by a thin cellular sheet formed by the choanocyte lateral appendages. The cytoplasm contains numerous clear vacuoles and some small metachromatic inclusions, less than 1 µm in diameter, which have the same ultrastructure as the smaller ones observed in granular cells. The collar is made up of thirty-eight or thirty-nine microvilli and is surrounded externally by a "glycocalyx" consisting of a reticulation of thin fibrils. The flagellum has no vane, but displays a sheet of fibrillar material similar to that observed outside the collar. No apopylar or cone cells were found, but their absence has to be checked on a larger number of chambers. The mesohyl has a low density around the choanocyte chambers, with a few extracellular symbiotic bacteria and highly dispersed collagen fibrils.

FIG. 5. — *M. araneosa* n.sp.. **A**, TEM of the sponge surface; **pi**, exopinacocyte; **os**, ostium (?); **B**, TEM of the sponge surface showing the pinacocyte layer with a cell junction (arrow), the cuticle (c), the "glycocalyx" layer, and a granular cell; **C**, TEM of the mesohyl showing elongate granular cells and a few extracellular bacteria; **D**, TEM of a small canal lined by an endopinacocyte; **E**, TEM of a canal lined by endopinacocytes and granular cells, one of which (left) contains numerous intracellular bacteria. Scale bars: A, 3.2 µm; B, 0.6 µm; C, 1.8 µm; D, 1.6 µm; E, 2.0 µm.


pi
os
A
c
B
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D
E

The intercellular matrix is made up of collagen fibrils with variable density. Fibrils are most often irregularly dispersed, except in zones with elongated granular cells and beneath the pinacoderm, where they form thin, poorly organized fascicles. Fibrils are of the rough type (Garrone 1978), 18-19 nm in diameter, with a poorly defined striation of approximately 22 nm periodicity. The matrix contains dispersed extracellular bacteria (Fig. 5C) which do not display the high morphological variety of the intracellular bacteria. Most are rod-shaped cells, 0.2-0.3/1-1.3 µm, with a dense area in the clear central nuclear zone.

Reproduction

No stages of reproduction has been observed.

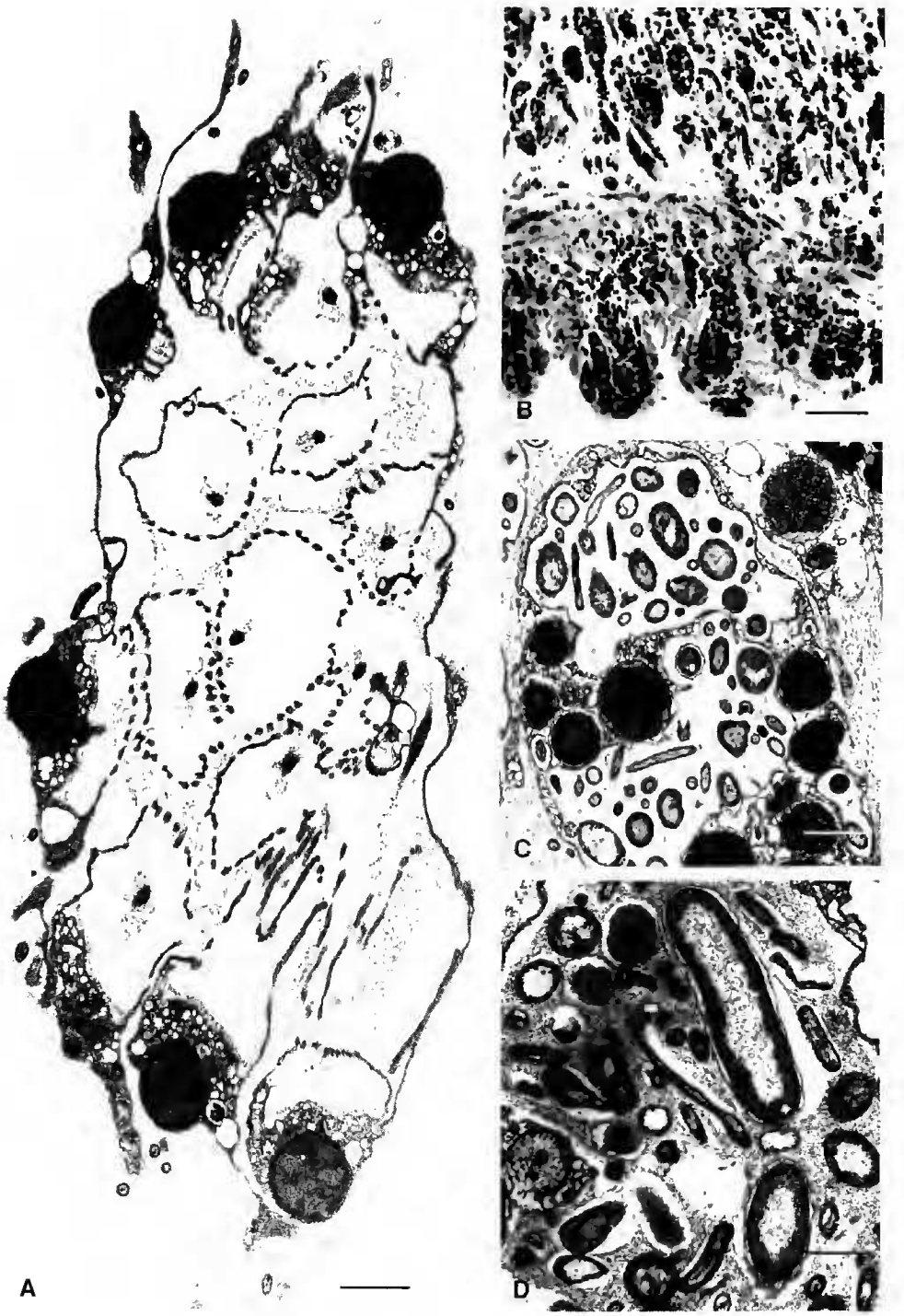
REMARKS

This sponge without skeleton is very unusual with regard to anatomy and cytology, with a remarkable uniformity of cell types, a reduced aquiferous system, and an unusual morphology and mode of growth. These peculiarities, which are observed year round in all specimens examined, are not related to stages of reproduction, which may temporarily change the anatomy and cytology of some demosponges such as *Halisarca* drastically (Lévi 1956; Chen 1976; Bergquist 1996). They are also not related to a possible tissue regression, which has been described in overwintering specimens or in sponges undergoing degeneration by fragmentation under adverse conditions. Tissue regression causes a reduction or loss of the aquiferous system and a dedifferentiation of most cell types (Simpson 1984), which frequently become archaeocyte-like cells with numerous residual bodies, thus resulting in features which are reminiscent of those observed in *Myceliospongia*. However, the stability of characters in all the examined specimens and our observations on the growth of *Myceliospongia* during a six months period (Fig. 4B, C) both indicate clearly that we have observed normal, non-degenerating sponges. The relative resemblance of the cell features to those described during tissue regression only indicates that the mesohyl cells are here constantly poorly differentiated.

The above characteristics do not permit the allocation of *Myceliospongia* to any known family of Demospongiae in which non-skeletonized genera have been described. The simplicity of the cytology is shared with the Homoscleromorpha, in which two genera without skeleton are known (*Oscarella* and *Pseudocorticium*). However, *Myceliospongia* does not display any of the unique characteristics of this subclass, such as flagellated exo- and endopinacocytes, large choanocyte chambers, and a unique basement membrane underlying both pinacoderm and choanoderm (Boury-Esnault *et al.* 1984 ; Boute *et al.* 1996). The histology and anatomy differ extensively from those of Chondrillidae and Darwinellidae, families in which the genera *Chondrosia* and *Hexadella* respectively are devoid of skeleton. The sponge is clearly distinct from the new genus of Chondrillidae, *Thymosiopsis*, described earlier in this paper. It has neither the ectosomal organization of collagen fibrils nor the tubular, branched choanocyte chambers which are distinctive of the order Halisarcida (Bergquist 1996). The possibly distinctive features shared in varying measure by species of Halisarcidae and of Chondrillidae, such as a granulo-fibrillar matrix distinct from the collagen zones in which the choanocytes are anchored by long pseudopodia (Vacelet & Donadey 1987), are absent. There is no possible relationship to *Bajalus* Lendenfeld, 1885 which has recently been shown to be a synonym of *Halisarca* (Bergquist 1996).

Furthermore, the unique characters of *Myceliospongia* provide no clear evidence which would permit affiliation with any existing order of skeletonized Demospongiae. The fibrillar layer covering the exopinacocytes resembles the "glycocalyx" of the poecilosclerid *Hemimycale columella* (Willenz 1982). The absence of typical spherulous cells and the large number of inclusions similar to residual bodies in most cell types are shared with the keratose sponge *Dysidea avara* (Uriz *et al.* 1996). However, no relationship between *Myceliospongia* and the genera

FIG. 6. — *M. araneosa* n.sp. **A**, TEM of a choanocyte chamber; note the lateral expansions of the choanocytes. **B**, semi-thin section through the mesohyl near the undersurface (bottom). **C**, TEM of a granular cell with numerous intracellular bacteria. **D**, intracellular bacteria in a granular cell. Scale bars: A, 1.3 µm; B, 22.5 µm; C, 1.6 µm; D, 1.0 µm.


A
B
C
D

Hemimycale and *Dysidea* should be suggested on the basis of these features alone. The only evidence is the rough nature of the collagen fibrils, which provides a weak indication of affiliation with the subclass Ceractinomorpha (Garrone 1978). The relationships of the genus within the Demo-spongiae thus cannot be resolved at present. The absence of morphological or cytological affinities with any order of Demospongiae could justify the distinction of a new order. However, we prefer to presently classify *Myceliospongia* as a genus *incertae sedis* within the Demospongiae, possibly wirhin the Ceractinomorpha, pending further biochemical, reproductive or genetic information.

Unlike most other sponges without skeleton, the absence of fibres or spicules is nor counterbalanced by a development of dense fascicles of collagen fibrils, as it is in Chondrillidae or Halisarcidae, or by a basement membrane as is the case in Homoscleromorpha. Consequently, the sponge is soft and fragile, and probably would not be able to withstand moderately exposed littoral environments.

The unique body organization, with a reduced aquiferous system, few choanocyte chambers, a remarkably developed system of expansions on the substratum which increases the external exchange surface, and a mucous sheet on the surface suggests that the sponge has a peculiar life strategy. Apparently, it is able to survive and even to grow expansions when the whole body is covered by massive sponges which smother most of the surface. It may be supposed that filaments have a role in the capture and ingestion of particles, with the mucous sheet possibly acting as in *Hemimycale columella* (Willenz 1981, 1982). Further studies are needed to elucidate how this sponge is functioning.

DISCUSSION

These two sponges are known only from the 3PP cave. This habitat is highly unusual in having stable temperature conditions, which approximate those of the deep Mediterranean which is homeothermic at 13 °C. It is quite unlikely that the sponges have been overlooked in other caves from the Marseille area, especially *Myceliospongia araneosa* n.sp. with its remarkable growth form. The cave has been submerged since the last Holocene sea level rise, 7000-8000 years ago, a relatively short time which would not permit local differentiation of such taxa. Consequently, the restricted known distribution to a cave which shelters a number of deep-sea invertebrates never recorded in other caves (Vacelet *et al.* 1994) implies that the two new sponges also live in the bathyal zone, from where they have colonized the cave. The 3PP cave is only 7 km distant from the Cassidaigne canyon, which is 100 to 3000 m deep with a poorly known invertebrate fauna. Although the sponge fauna of the canyon has been thoroughly explored (Vacelet 1969), the numerous precipitous walls and downward-facing surfaces certainly shelter many unrecorded sessile invertebrates which are very difficult or impossible to sample and observe. It is likely that propagules of the bathyal invertebrates present in the 3 PP cave have been advected by an intermittent, strong upwelling current frequent in this area (Bourcier 1978; Millot 1979). These invertebrates are absent from other littoral aphotic environments because of their stenothermic character.

The two new sponges significantly increase the already remarkably high number of sponges without any skeleton which live in dark caves. This remarkable abundance, which is probably shared by bathyal vertical and overhanging surfaces as discussed above, is not clearly related to any characteristic of this environment. It has been hypothesized that in karstic cave, a possible reduced amount of silica in the water prevents some demosponges from fully developing their spicule skeleton, thus explaining a relatively high frequency of spicule abnormalities (Bibiloni & Gili 1982; Bibiloni *et al.* 1989). However, it has been shown (Fichez 1989) that in a karstic cave, the amount of silica increases from the entrance to the terminal part, as a result of mineralization processes during the residence time of the water in the cave. It appears more likely that the extremely low hydrodynamic energy experienced by most dark caves and deep-sea environments allows the development of fragile invertebrates, such as sponges without skeleton or without a

highly developed collagen cortex, which could not withstand exposed littoral environments.

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